Hypertension and Insulin Resistant Models Have Divergent Propensities to Learned Helpless Behavior in Rodents

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The induction of learned helpless (LH) behavior in rats is a widely used model of unipolar depression. Recent studies have linked depression with hypertension and insulin resistance as observed in obesity, but the propensity of these disorders to manifest depression has not been reported. In this study, the LH behavioral paradigm was exploited in a model of hypertension (Dahl rat) and of insulin resistance (Zucker rat) to determine the propensity of these models to develop depression and to examine the profile of markers for the propensity of the cardiovascular system (plasma renin activity) and of the hypothalamus–pituitary–adrenal axis (corticosterone) in the display of propensity to depression. Results show that Zucker rats displayed the lowest propensity to the development of LH behavior (12%), followed by the control Sprague-Dawley rats (27%), and then Dahl rats (66%). In contrast, congenital learned helpless (cLH) rats, a genetically bred strain for animal depression, had the highest propensity (>90%). A gender effect was observed in the Zucker and cLH rats, with females showing an increased propensity to develop LH behavior. Plasma renin activity in the Dahl and Sprague-Dawley rats after the LH stress paradigm was not significantly different from baseline. In contrast, Zucker rats, with the lowest propensity to LH behavior, demonstrated a threefold increase in plasma renin activity after stress. Congenital LH rats, with the highest propensity to LH behavior, exhibited a significantly lower increase (43%) in plasma renin activity after stress. Hyporesponsive hypothalamus–pituitary–adrenal (HPA) axis functioning correlated with propensity of LH behavior. Stress-induced corticosterone levels increased under twofold in cLH rats, whereas they increased more than sevenfold in Zucker rats. Taken together, these studies suggest that whereas genetically prone hypertensive rats have a very high propensity to depression, insulin-resistant rats have a profound resistance to depression. Moreover, a hyporesponsive HPA axis may be a marker for disorders that are comorbid with depression, whereas a hyperresponsive renin-angiotensin system may be indicative of resilience. Am J Hypertens 2000;13:659–665 © 2000 American Journal of Hypertension, Ltd.

KEY WORDS: Hypertension, insulin resistance, depression, comorbidity, corticosterone, renin-angiotensin system, congenital learned helplessness, Zucker rats, Dahl rats.

Development of hypertension and insulin resistance in the Dahl and Zucker rats, respectively, is a multifactorial process involving interactions of environmental and genetic factors.1–3 A complex interaction between environmental factors and genetic predisposition has also been suggested in the development of human hypertensive disease and insulin resistance.1,5 Recently, a study from the National Center for Health Statistics, Centers for Diseases Control and Prevention presented strong evidence linking stress with hypertension, insulin resistance, and depression.6 For exam-
ple, hypertension negatively impacts information processing, memory, and anxiety states.7 Conversely, anxiety and depression are predictive of later incidence of hypertension.6,8 Although the relationship between obesity and emotionallity has been more tenuous,9 various studies have demonstrated a strong association between high fat content and inadequate stress handling.10 These unusual associations prompted the possibility that common neurophysiologic mechanisms may subserve the behavioral, neuroendocrine, and cardiovascular abnormalities in the Zucker and Dahl rats and suggested that perhaps these animals would have a higher sensitivity to develop depression than normal animals.

Induction of learned helpless (LH) behavior in animals has been widely used as a model of depression.11–15 Rats exposed to uncontrollable footshock subsequently develop performance deficits or LH behavior in a shock escape test and this phenomenon has been advanced as a model of animal depression.13,14 Both LH behavior and depression are induced by environmental and psychosocial events.16,17 The LH behavioral outcome of various parameters such as weight loss, sleep activity, libido, and cognitive function are remarkably similar to depression.18–20 Furthermore, identical pharmacologic treatments are effective in improving both conditions induced by environmental and genetic factors.21–23 We, therefore, reasoned that Zucker and Dahl rats would have a higher propensity to demonstrate LH behavior and that changes in the renin–angiotensin system (a measure of cardiovascular system functioning) and in corticosterone (a measure of the hypothalamus–pituitary–adrenal axis functioning) would be associated with the higher propensities. In addition to identifying the differing propensities, the present data have identified that an impairment in the hypothalamic–pituitary–adrenal (HPA) axis may be a marker for clinical disorders comorbid with depression and that the renin–angiotensin system may be a possible marker for decreased susceptibility.

METHODS

Animals Age-matched congenital learned helpless (cLH), Zucker, Dahl, and Sprague-Dawley rats (males and females, 250 to 300 g) were obtained from our LH breeding colony at the University of Maryland (Baltimore, MD) and from commercial breeding laboratories (Harlan Sprague Dawley Laboratories, Indianapolis, IN, for the Zucker and Dahl rats and Charles River Laboratories, Wilmington, MA, for the Sprague Dawley rats). The animals were housed three to a cage in a humidity- and temperature-controlled facility (50 ± 10%; 23 ± 1°C) under a 12-h light/dark cycle (7 AM/7 PM). Food and water were available continuously. All protocols in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland. Behavioral experiments and all sample collection were carried out between 8 and 11 AM.

Learned Helplessness Paradigm and Breeding The established paradigm for helplessness training and testing has been previously described.13 Briefly, training consists of giving rats intermittent and random footshock through an electrified grid floor at 0.8 mA for 40 min (Coulbourn solid state shock source; model E 13-16, Coulbourn Instruments, Allentown, PA). Twenty-four hours later, the rats were tested in a shock escape paradigm where footshock can be eliminated by a single bar press. Failure to eliminate the shock within 20 sec was recorded as a failed trial. Fifteen trials were given during each testing session and intertrial latency was set at 45 sec. Behavioral deficits were measured as the mean number of failures (± SEM) using the following criteria: rats scoring 0 to 5 failures in a 15-trial testing session were nonlearned helpless (NLH) and do not differ from naive control rats; borderline-learned helpless rats scored 6 to 10 failures; and learned helpless rats scored 11 to 15 failures a trial testing session. Full sib pairing within the LH and NLH groups resulted in successive generations of two selective strains: the cLH strain, uniquely susceptible to the development of learned helpless behavior, and the cNLH strain, resistant to the development of the same behavior.24

Zucker-fa Rat Model of Obesity and Insulin Resistance The HsdHlr Zucker-fa rat was developed from a colony maintained at Hoffman-La Roche in 1992.25 A mutation at the fa locus results in the onset of obesity as early as 3 weeks of age. The Zucker-fa rats used in the present study were purchased from Harlan Sprague Dawley Laboratories (Indianapolis, IN) where the stock is maintained by mating heterozygote male and female rats of the strain. The Zucker-fa rat differs from littermate controls by body weight, increased fat cell proliferation, and by insulin resistance.26

Dahl Salt-Sensitive Rat Model of Hypertension The Dahl salt-sensitive rats (SS/JrHsd) were developed from a colony of Sprague Dawley rats maintained at the Medical College of Ohio in 1986.27,28 These rats remain normotensive unless they are exposed to a high salt diet. Although the strain was originally developed by selective inbreeding, the genetic status of the line was changed by the introduction of heterozygous stocks into the line. The rats used in the present experiments were purchased from Harlan Sprague Dawley Laboratories (Indianapolis, IN), where a bidirectional selection for the predisposition to salt-induced hypertension is continued.
Sprague-Dawley Rat The Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, MA). In the present experiments, these rats serve as outbred controls.

Corticosterone and Renin Analysis Rats from each strain (cLH, Zucker, Dahl, and Sprague-Dawley; n = 30/strain exposed or not exposed to the LH paradigm) were sacrificed 2 h after the shock escape testing. Trunk blood for measurement of corticosterone and plasma renin activity was centrifuged at 5000 g at 4°C for 15 min. Aliquots of plasma samples were kept frozen at −80°C until the assays were performed. Measurement of corticosterone was performed in 1 ml unextracted plasma, and run in duplicate. The corticosterone assay (ICN, Costa Mesa, CA) has a sensitivity of 0.5 ng/dL, with 10% and 7.5% inter- and intraassay coefficient of variation, respectively. Plasma renin activity (PRA) was measured using a commercially available source (RIIANEN angiotensin I, Dupont, Boston, MA). The specificity of the assay is approximately 30 pg/mL/h. The specificity of the assay is 100% cross reactivity for angiotensin I, 0.6% for angiotensinogen, and less than 0.006% for angiotensins II and III. Values are reported as nanograms of angiotensin I generated per milliliter of plasma per hour of incubation.

Statistical Analysis Statistical assessments were made by ANOVA, with correction for multiple comparison where appropriate, and Dunnett’s test with a statistical package.

RESULTS

Learned Helpless Behavior: Effects of Strain and Gender After exposure to uncontrollable shock, the response of cLH, Zucker, Dahl, and Sprague-Dawley male rats in a subsequent shock escape test was evaluated. Figure 1 details the features of the escape response in these rats. In each panel, populations of rats are classified as follows: response deficient or learned helpless (LH) when they scored 11 to 15 failures in the 15-trial shock escape test; nondeficient or nonlearned helpless (NLH) when they scored 0 to 5 failures in the 15-trial shock escape test; and borderline when they scored 6 to 10 failures in the same test. A total of 30 rats were used for each strain. Figure 1A shows the shock escape profile of the Sprague-Dawley control rats: 27% of these rats scored in the 11 to 15 failure range, 31% scored in the borderline or 6 to 10 failure range, and 42% scored in the 0 to 5 failure range, suggesting that none of the cLH rats were resistant to learned helpless behavior. Figure 1B illustrates the shock escape profile of the cLH rats: 88% of these rats tested scored in the 11 to 15 failure range, 12% of scored in the borderline or 6 to 10 failure range, and none of the cLH rats score in the 0 to 5 failure range, suggesting that none of the cLH rats were resistant to learned helpless behavior. Figure 1C illustrates the shock escape profile of the Zucker rats: 12% of these rats tested scored in the 11 to 15 failure range, 31% were borderline, and 57% scored in the 0 to 5 failure range, suggesting that the majority of the Zucker rats were resistant to LH behavior. Figure 1D illustrates the shock escape profile of the Dahl rats: 66% scored in the 11 to 15 range, 17% in the 6 to 10 borderline range, and 17% in the 0 to 5 resistant NLH range, suggesting that the majority of these animals exhibited LH behavior. Figure 2 shows that cLH, Zucker, Dahl, and Sprague-Dawley female rats exhibited a different profile from males in the shock escape test. This was most evident in the cLH and the Zucker rats were gender effect was most noticeably demonstrated (cLH females: 94% LH, 0% NLH, and 6% NLH; Zucker females: 38% LH, 31% borderline, and 31% NLH). Thus, whereas Zucker males were 12% LH, females were 38% (Figure 2).

Plasma Corticosterone Figure 3 summarizes the effect of the LH stress paradigm on plasma corticosterone (CORT) levels in cLH, Zucker, Dahl, and Sprague-Dawley control rats (n = 30/strain). Unstressed cLH, Zucker, and Sprague-Dawley rats had comparable basal CORT levels (ng/mL ± SEM: 5.7 ± 0.3, cLH; 3.7 ± 0.5, Zucker; 4.1 ± 0.9, Sprague-Dawley). However, Dahl rats exhibited a threefold increase in baseline CORT levels (ng/mL ± SEM: 15.2 ± 0.6, SE: 15.2 ± 0.6).
P.001 compared to the other strains). Stress significantly increased CORT levels in all strains: cLH two-fold increase; Dahl, threefold increase; Sprague-Dawley, sixfold increase; and Zucker, sevenfold increase in CORT concentrations (P < .01; Figure 3). There was thus an inverse relationship between HPA axis responsivity and propensity to LH behavior. Therefore, the overall effect of stress on the activity of the HPA axis was significantly lower in the cLH rats compared to all other strains examined, as assessed by plasma CORT concentration (Figure 3).

**DISCUSSION**

The most striking, and surprising, observation in this study is that animals genetically prone to hypertension have a very high propensity to develop depression, whereas those prone to insulin resistance have a high propensity to resilience. Although most studies have focus on insulin resistance in depression\(^2\)\(^9\),\(^3\)\(^0\) and hypertension in depression,\(^3\)\(^1\),\(^3\)\(^2\) few have focused on the propensity of depression in hypertension and insulin resistance. The present results agree well with reports for human populations showing that the presence of depression is a risk factor for cardiovascular and cerebral events, but for which the mechanism remains obscure. For hypertension, it has been suggested that enhanced platelet activity observed in depression may be an initiating factor,\(^3\)\(^2\) but details as to how such a mechanism might work was not provided. The enhanced propensity to learned helplessness observed in hypertension was associated with a sup-
pressed PRA (see Figure 4). Blockade of the renin–angiotensin system with the angiotensin I-converting enzyme inhibitor perindopril reversed the learned helplessness in rodents, prompting the suggestion that the renin–angiotensin system may not only participate in the elevation of blood pressure but also contribute to the depression. For insulin resistance, it has been suggested that activation of the HPA axis and the central sympathetic nervous system is responsible not only for the insulin resistance but also for other disorders, including hypertension. Insulin resistance, hypertension, and depression are strongly genetic disorders.

The results of the present study demonstrate that various genetic strains of Sprague-Dawley rats show different propensities to depression, as assayed by the divergent behavioral profile in the LH stress paradigm. Recently, twin, adoption, and family studies have suggested that genetic factors are important in the etiology of mood disorders. We have also established a strong correlation between the LH phenomenon and genetic influences as after several generations of selective breeding within our LH model, the cLH strain emerged and exhibited an increased vulnerability to helplessness even in the absence of prior exposure to uncontrollable stress. In addition to the cLH strain, another strain was concurrently bred to exhibit a relative resistance to helpless behavior. The interesting finding of the very high propensity of the hypertensive rats to develop LH behavior in contrast to the very low propensity of the obese insulin resistant rats (with the Dahl rats showing a fivefold greater propensity over the Zucker) prompts numerous speculations, although the underlying physiologic mechanism responsible for this disparity is unknown. Recent studies have suggested hypercorticism to be a key factor in the obesity syndrome of the Zucker (fa/fa) rat. As Zucker rats grow and develop obesity, a significant difference in pro-opiomelanocortin (POMC) gene expression mRNA has been demonstrated when compared to lean control rats. These elevated POMC mRNA levels are at least partially responsible for the increased adrenocorticotropic hormone and corticosterone in the adult fatty rats and elevated endogenous corticosterone levels have been implicated in the maintenance of hypertension in obese Zucker rats. In addition, a gender effect was only evident in two of the strains examined, with the female cLH and Zucker rats showing an increased propensity to LH behavior. The physiologic basis of the gender disparity is unknown.

Our data also uncover an interesting inverse correlation between propensity to LH behavior and stress responsivity, as measured by corticosterone levels. Baseline plasma corticosterone levels were similar in the LH, Zucker, and Sprague-Dawley rats, whereas these levels were higher in the Dahl rats. However, LH rats display a blunted stress response after shock exposure in the LH paradigm. These rats only exhibited a 59% increase in corticosterone levels compared to 215%, 456%, and 653% increase in the Dahl, Sprague-Dawley, and Zucker rats, respectively. Previous data have shown that the cLH rats have a differential response to various stressors when compared to congenital nonlearned helpless and Sprague-Dawley rats. This differential response to stressors is interesting and of potential significance as the cLH animals represent a widely used genetic model for anxiety disorder and animal depression. We have also previously demonstrated that glucocorticoids influence the induction of LH behavior as both adrenalectomy and treatment with RU 38486 enhance the development of the behavior. The cLH rats used in the present studies, inherently exhibit similar behavioral responses in the LH paradigm in the absence of surgical or drug manipulations. The increased vulnerability exhibited by the cLH rats is correlated with alterations in glucocorticoid-mediated gene expression in limbic structures.

Hyperactivity in the renin–angiotensin system was positively correlated with a decreased susceptibility to depression, as monitored by the LH behavior. In the cLH rats, a fairly convergent profile was demonstrated for the renin–angiotensin system and HPA axis. Baseline and stress-induced PRA levels were significantly lower in cLH rats compared to resistant controls. When compared to the Zucker, Sprague-Dawley, and Dahl rats, basal PRA was not significantly different in the cLH rats. In response to stress, however, PRA was unchanged in the Sprague-Dawley and Dahl rats, but it increased by 43% in the cLH rats and by 186% in the Zucker rats. This activation of the cardiovascular system may stem from active coping mechanisms in these rats because there was an inverse relationship between cardiovascular reactivity and propensity to LH behavior, suggesting that genetic vulnerability may play an important role in the physiologic and behavioral responsivity of stress responsive systems. The impact of these factors on the pathogenesis of cardiovascular and diabetic disorders may have implications not only for therapy but also for diagnoses.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of S. Adolphe in the preparation of this manuscript.

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